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Anthocyanin synthesis and light utilisation can be enhanced by reflective mulch – Visualisation of light penetration into a tree canopy

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ABSTRACT

With the increasing use of hailnets and decrease in light availability in the ripening period of apple fruit, insufficient light exposure often causes poor colouration viz anthocyanin synthesis on certain parts of the fruit and on certain fruit within the tree. The aim of this study was to investigate the potential of improving anthocyanin synthesis, in terms of fruit colouration, the major incentive for a consumer. Therefore, the reflective ground cover Lumilys® was spread between 'Braeburn Mariri Red' apple rows under a crystalline hailnet seven weeks prior to harvest and colour measured on 240 attached apple fruit. The reflective ground cover increased the reflected light by 1.6 to 3.9 times 1.0 m above ground. The improved light utilization led to an improved peel colouration, especially on the shaded side of the apple fruit and fruit in the lower inner part of the canopy, A coloured visualization from orange (high light intensity), yellow (medium) to green (low light intensity) as a result of the individual PAR measurements every 20 cm inside the canopy showed how the reflective mulch influences the light penetration into the different parts of the tree canopy.

1. Introduction

Anthocyanin biosynthesis in the fruit peel is regulated through MYB 10 by temperature (minimum temperature and day and night difference) and light (daylength/photoperiod, light intensity and solar angle). All three light features decline in autumn, resulting in a reduction of both temperature differentials and radiation necessary for anthocyanin biosynthesis; this light deprivation is further accentuated under hailnets with 8% to 22% light loss (Solomakhin and Blanke, 2007). Since the fruit colour is the major incentive for consumer acceptance (Blanke and Kunz, 2016), enhanced anthocyanin synthesis particularly of poorly coloured fruit or their shaded, less coloured side facing the tree trunk is desirable. Methods to enhance the fruit colouration include a) reflective ground cover, b) chemical colour enhancers and c) to a lesser extent, evaporative cooling, which requires access to water (Blanke, 2015). When spread in the alleyway during fruit maturation, reflective ground covers can increase light (PAR, UV) reflection (Hjeltnes et al., 2004) and hence interception of the fruit (Funke and Blanke, 2005; Hanrahan et al., 2008; Meinhold et al., 2011).

The first objective of this study was to determine the physiological effects of reflective mulches (Lumilys®) on fruit quality with anthocyanin synthesis i.e. colouration in apple cv. 'Braeburn Mariri Red'. Attached fruit from different positions within the canopy were selected and four sides of these fruit examined. The second goal was to examine the light reflection by the mulch and light penetration into different parts of the tree canopy, as affected by the presence or absence of reflective mulch and visualise the results using a colour scheme.

2. Material and methods

The climate at Klein-Altendorf (50 °N) is Atlantic, predominantly with Westerly winds. Five-year-old apple trees (*Malus domestica Borkh.*) of the bi-coloured cultivar 'Braeburn Mariri Red' on M9 rootstock were planted in north-south orientation with a spacing of 3.5 m x 1.0 m and trained to slender spindles either in the open field or under a pearl hailnet with a mesh size of 3 mm x 8 mm with or without a white textile ground cover (Lumilys®). Each treatment consisted of ten trees as replicates. The reflective ground cover Lumilys® type WH 100 (polypropylene, 100 g/m^2; 2.6 m wide; Beaulieu Technical Textiles, Waregem, Belgium)(Beaulieu Technical Textiles, 2016) leaves a gap of 0.9 m for precipitation, soil and grass aeration (Fig. 1) and was spread on 5 September 2016 for seven weeks until harvest.

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Fig. 1. Reflective ground cover Lumilys® (5 Sept. 2016) on both sides of the apple trees under hailnet in Klein-Altendorf, secured with sand bags.

2.1. Light measurements

Light was measured every hour from 8 a.m. to 6 pm on an overcast autumn day in the orchard with an EGM-5 Portable CO_2 Gas Analyzer with a TRP-3 (CS 1) both from PP Systems, USA over mulch and over grass. Incident light was measured in PAR (400–700 nm) after the international standard with the sensor facing upward, 1 m above ground in the middle of the grass alley (Funke and Blanke, 2011) facing south. Reflectance was measured with the sensor facing downward and in a 45° angle on the eastern side of the tree row (Fig. 2).

Within the tree canopy, light intensity was measured as PAR three times at each spot, with an upward facing sensor, with a downward facing (45° and 90°) sensor (Fig. 2b) one hour before and one hour afternoon i.e. the solar zenith. Light values were taken 200 mm apart horizontally starting at the tree trunk at heights of 500 mm; 1000 mm; 1500 mm and 2000 mm above the ground, on the eastern and western side of the tree trunk both with and without the reflective ground cover Lumilys® using the method derived from Cohen et al. (1995). Light intensity values inside the canopy were visualised using colour-coding with 5% increments as percentages relative to the PAR values outside of the canopy in the middle of the alleyway (Fig. 2a), using standard Excel and its colour scheme. The largest PAR values in the tree periphery were displayed in orange, the intermediate values in yellow tones and the low light intensities in green tones. Altogether, 20 colours were

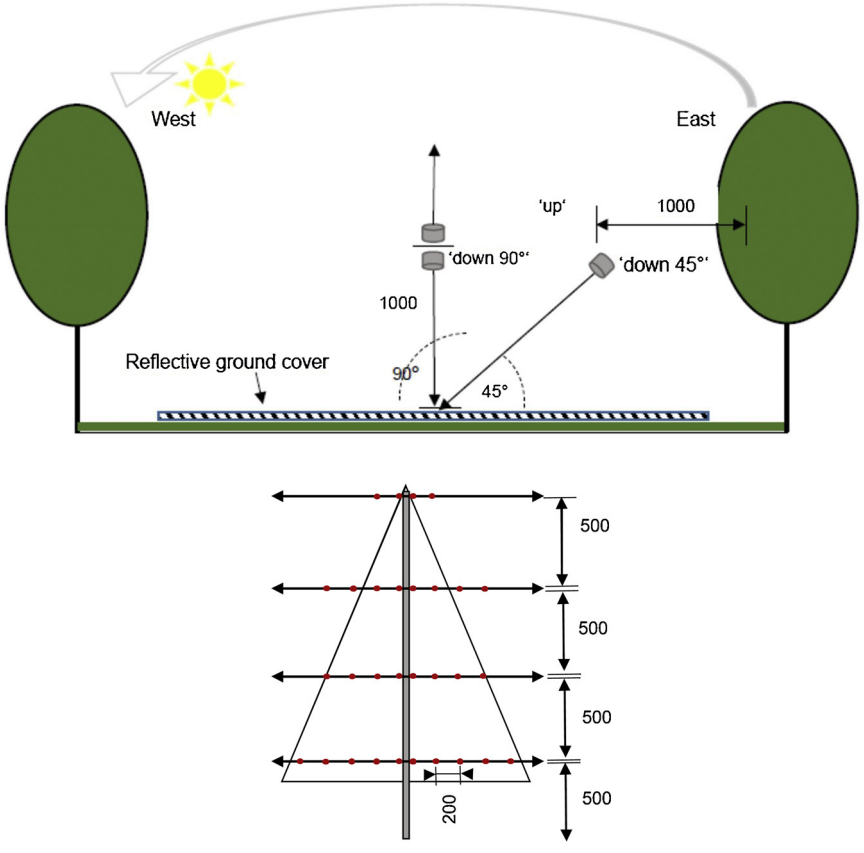

Fig. 2. Scheme of a) light reflection measurements over a reflective ground cover or a grassed alleyway in Klein-Altendorf in 2016 and b) the measurement points for the PAR intensity measurements inside the canopy at four heights and five distances from the tree trunk on the eastern and western side of the tree (distances in mm).

employed to visualize the relative light intensities within the tree canopy in steps of 5%.

2.2. Fruit quality assessment

To differentiate between the fruit quality in the different parts of the tree canopy, apples were sampled at harvest from the western and the eastern side of each tree and from the inner and outer part of the lower and middle part of the canopy. The colour of these 240 apples was measured on four sides every 90° angle around the fruit equator using the CIE L*, a*, b* colour space with an i1PRO (X-rite, USA) and hue colour angles calculated (McGuire, 1992).

2.3. Statistical analysis

Data were subjected to statistical analysis using the program IBM® SPSS® Statistics (Version 24, IBM, USA) using the Shapiro-Wilks test for normal distribution and the Kruskal Wallis test with Dunn-Bonferroni-Tests as a pairwise comparison.

3. Results

3.1. Light reflection in the orchard as affected by the reflective ground cover

Light was measured as PAR on an overcast day (11 Oct. 2016), with and without a hailnet and with and without a reflective ground cover, when PAR underneath a pearl hailnet started from 25 µmol m^{-2} s^{-1} (incident) and 11 µmol m^{-2} s^{-1} (90°) with reflecting ground cover (Fig. 3a). While the day was partly sunny, irradiation reached it´s peak with almost 1100 µmol m^{-2} s^{-1}.

Until 18 h, PAR decreased to 43 µmol m^2 s^{-1} from above and 25 µmol m^{-2} s^{-1} reflected from the ground cover. Unexpectedly, the proportional light reflection on the overcast day of 30%–70% slant reflection and 20%–50% straight reflection was larger than on the sunny day (result not shown). In the grassed alleyway, reflected PAR in the foggy morning started with ca. 5 µmol m^{-2} s^{-1}. While the straight reflection peaked at 71 µmol m^{-2} s^{-1} at 14 h, slant reflection reached 228 µmol m^{-2} s^{-1} at 15 h, when the sun came out, both values decreased to ca. 15 µmol m^{-2} s^{-1} in the evening.

Slant reflection from the ground cover without a hailnet increased from ca. 20 µmol m^{-2} s^{-1} to 520 µmol m^{-2} s^{-1}, while the straight reflection reached 633 µmol m^{-2} s^{-1} when the sun was shining from 13 to 15 h and both decreased to about 30 µmol m^{-2} s^{-1} in the evening (Fig. 3b). Proportional reflection in the morning decreased from 50% - 60% to ca. 30% at 14 h. Slant reflection increased up 90% and up to 60% in reflected straight. The grass reflected only 10%–30% of the incident light in the morning (4–9 µmol m^{-2} s^{-1}). Reflected light peaked at 503 µmol m^{-2} s^{-1} (45°), when the low sunbeams entered the alleyway on the sunny day, representing almost 90% of incident light on an overcast day and 152 µmol m^{-2} s^{-1} (90°) representing almost 30% at 15 h.

3.2. Light intensity in the canopy as affected by a reflective ground cover

Fig. 4 visualises the light distribution using colour codes from orange (high light), yellow to green (low light intensity) in the tree canopy with (Fig. 4b, d, f) and without (Fig. 4a, c, e) a reflective ground cover underneath a hailnet one hour before and after the highest solar position (39°) in the middle of October. A comparison of the light intensity in the canopy with and without the reflective ground cover, shows an increase in slant and straight reflected light, indicated by lighter green and yellow tones. Low light intensities dominate, indicated by darker green colours, irrespective of employing reflective mulch in the inner and lower part of the tree canopy 1 m above ground.

In the tree canopy without reflective ground cover, the largest difference concerning the light intensity in the tree canopy could be seen, when the sensor is held upwards (Fig. 4e, f), where intense green colour implies less light penetration into the canopy. Interestingly, the large bright areas inside the tree canopy, measured with the sensor held upwards (Fig. 4f), may be explained by repeated reflection from the reflective ground cover and then from the *abaxial* leaf, which exhibits a white to light green colour compared to the dark green *adaxial* side of the leaf.

3.3. Effects of the reflective ground cover on fruit colouration

The average hue colour angle of the apples over the reflective ground cover decreased from 39° to 30° In the lower, inside of the canopy (close to the reflective mulch) compared with the untreated control (Fig. 5a). Overall, the reflective ground cover enhanced fruit colouration relative to the control; this was especially apparent in the lower inside of the canopy.

On the sun-exposed side of the apple fruit, no statistical significant differences could be detected between the variants in the middle and the lower outside of the canopy (Fig. 5b). A comparison of the fruit colouration between the positions in the canopy within one treatment on the sun-exposed fruit side shows that in the control group and the apples over the reflective ground cover the apples in the inside of the canopy showed higher hue colour angles, ranging between 25° and 27°, than the apples in the outside of the canopy with hue values ranging between 21° and 22°. This implies that overall the apples in the outer canopy were more red than in the inside. In this treatment apples of the

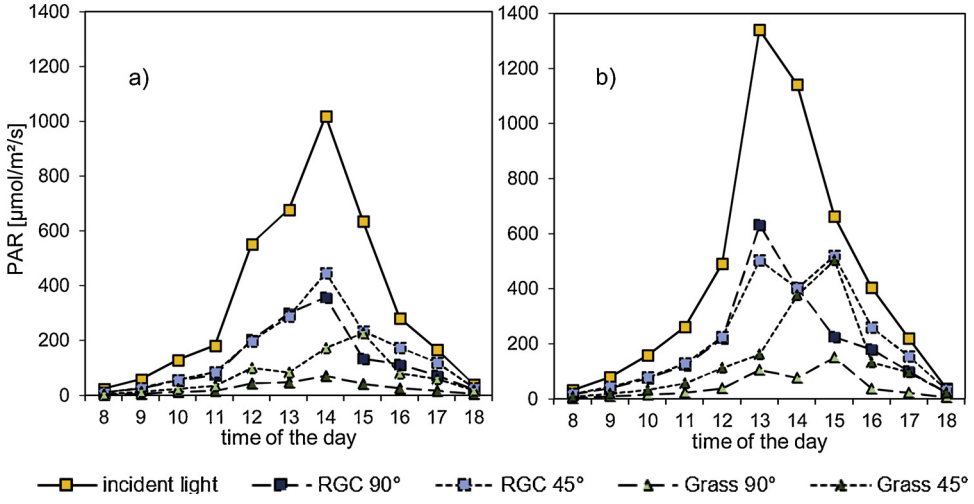

Fig. 3. Incident (yellow) and reflected light (in PAR) of the reflective ground cover (RGC) in a 90° (dark blue) and 45° angle (light blue) and of a grassed alleyway in a 90° (dark green) and 45° angle (light green) over the course of an overcast day with (a) and without (b) a hail net (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

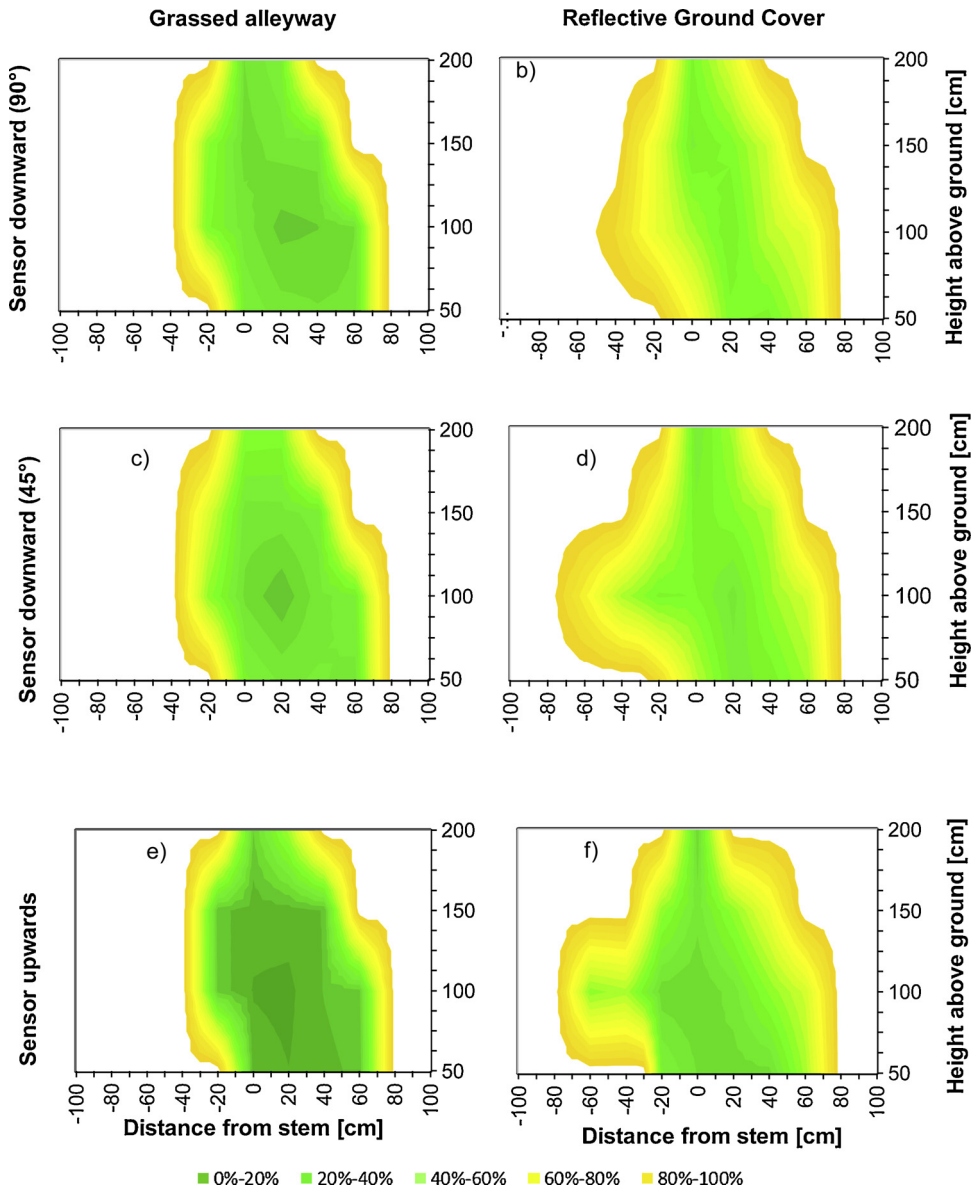

Fig. 4. Light penetration into the canopy measured on the 16.10.2016 with PAR in percent of the outside radiation with the sensor held downwards in a 90° (a and b) and a 45° angle (c and d) and upwards (e and f) in a tree with a grassed alleyway (control; left) and a tree with reflective ground cover (right) underneath a hailnet one hour before the highest solar position.

middle outside were the reddest, followed by lower outside and middle inside, while the apples in the middle inside showed the least amount of red colouration with a hue value of 34°.

Colouration of the shaded side of the apples over the reflective ground cover Lumilys® generally showed a significantly lower hue colour angle, especially for the lower inside of the canopy with 35° than untreated apples (55°) (Fig. 5b). A comparison of the hue colour angles in the four different canopy positions of the shaded side of the fruit within the control and Lumilys® shows the most progressed colouration in the outside of the canopy, the least progressed in the middle inside while the hue colour angles of the lower inside lay between them.

4. Discussion

This study aimed at investigating the physiological implications of reflective mulch on tree physiology viz light penetration to enhance anthocyanin synthesis viz fruit colouration.

4.1. Light reflection

The ground cover Lumilys® reflected up to 30% more light relative to the grassed alleyway on the overcast day, typical of autumn weather at 50 °N, when the additional light is needed the most (Blanke and Kunz, 2009; Schuhknecht et al., 2018); this is in line with Vercammen's study (2014) on Lumilys® in Belgium at the same 50° latitude, who measured an estimated 35% of reflected PAR on an overcast day and about 25% on a sunny day.

Solomakhin and Blanke (2007) explained this with the higher incidence of diffuse light on overcast days (Fig. 3) and found 19–20 % straight reflection, respectively, and 13–16 % scattered reflection underneath a hailnet on a sunny day at the same site, which equals a light improvement of about 12% compared to the reflection of the grassed alleyway. On the overcast day, Extenday™ respectively Daybright™ improved light reflection by 11–16 %, while Lumilys® had increased light reflection by 23–29 %(Solomakhin and Blanke, 2007).

Table 1 shows that in this study the light loss for anthocyanin synthesis through the use of hailnets ranges from no light loss at all to

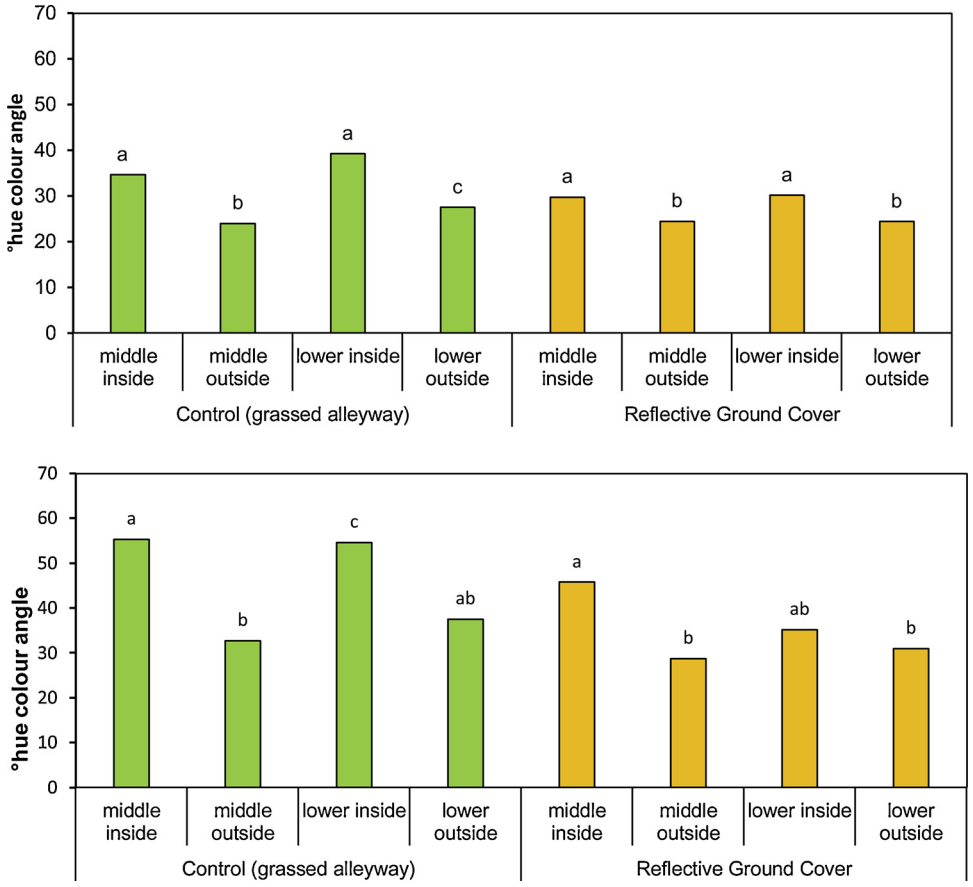

Fig. 5. hue colour angles of the ***sun-exposed (top)*** and of the ***shaded side (bottom)*** of apple fruit from the control (green) and over the reflective ground cover (yellow) in four different parts of the canopy of Braeburn apple trees in 2016 (median (n = 20)) in Klein-Altendorf in 2016. Different letters indicate significant differences at $p \leq 0.05$ between the treatments within one position in the tree canopy (Kruskal-Wallis with Dunn-Bonferroni) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

5% on the sunny day and 2% to 10% on the overcast day. The case of no light loss can be explained by the high proportional reflection of over 60 respectively over 40% in the evening, which was caused by the low position of the sun. Solomakhin and Blanke (2007) found slightly lower values with hailnets over the reflective ground cover Extenday™ and Daybright™ with the reduction of light reflection ranging from no light loss at all to a maximum of 5% on a sunny day and 0 to 3% on an overcast day. When the incident PAR is considered, the pearl hailnet causes an average light loss of 17.5% on a sunny day and 21.8% on an overcast day (Table 2).

4.2. Visualization of light penetration into the canopy with reflective mulches

The visualization of light distribution within the tree canopy (Fig. 4) shows that the increase in available light by the reflector is especially pronounced in the inner lower part of the tree canopy, where otherwise the green fruit dominate, possibly due to repeated reflection from the white mulch and then the lighter green-white abaxial leaf (Fig. 5).

Jackson and Palmer (1972) were the pioneers to investigate the light distribution in an apple orchard along apple tree rows in East Malling at 51 °N before computer and reflective mulches became available. To our knowledge, Privé et al. (2008) are the only authors, who measured light intensity in the apple tree with reflective mulch (Extenday™) in Canada; their light sensor was placed 0.5 m in the lower canopy of large trees (ca. 3.5 m high) and their results showed 5-fold to 9-fold greater reflected light at this lower height below the lowest fruit on the tree.

Our coloured visualization in Fig. 4 from orange (high light intensity), yellow (medium) to green (low light intensity) as a result of the individual PAR measurements inside the canopy showed how the reflective mulch influences the light penetration into the different parts of the tree canopy which has not been shown before to our knowledge.

4.3. Colour formation and fruit quality in different parts of the canopy

Overall, the increased light intensity in the orchard through the reflective ground cover Lumilys® stimulated the anthocyanin synthesis

Table 1
Mean reflected PAR in percentage of incident PAR of grassed alleyway and the reflective ground cover Lumilys® on a sunny (10.09.2016) and an overcast day (11.10.2016) in 2016 in Klein-Altendorf.

Weather	Treatment		Reflected PAR (90°)	Reflected PAR (45°)
sunny day	Grassed alleyway	Without hailnet	12 %	25 %
		With pearl hailnet	9 %	25 %
	Lumilys® textile mulch	Without hailnet	35 %	43 %
		With pearl hailnet	35 %	38 %
overcast day	Grassed alleyway	Without hailnet	12 %	36 %
		With pearl hailnet	10 %	26 %
	Lumilys® textile mulch	Without hailnet	46 %	57 %
		With pearl hailnet	39 %	49 %

Table 2
Reflected PAR in percentage of incident PAR of grassed alleyway and the reflective ground cover Extenday™ and Daybright™ on a sunny (05.09.2006) and an overcast day (06.09.2006) in 2006 in Klein-Altendorf as measured by Solomakhin and Blanke (2007).

Weather	Treatment		Reflected PAR (90°)	Reflected PAR (45°)
sunny day	Grassed alleyway	Without hailnet	4 – 5%	6 – 7 %
		With white/translucent hailnet	7 %	4 %
	Daybright™/Extendy™	Without hailnet	22 – 24 %	17 %
		With white/translucent hailnet	19 – 20 %	13 – 16 %
overcast day	Grassed alleyway	Without hailnet	4 – 5%	6 – 7 %
		With white/translucent hailnet	3 %	7 %
	Daybright™/Extendy™	Without hailnet	24 – 26 %	23 %
		With white/translucent hailnet	23 – 24 %	20 – 21 %

and therefore led to an improved fruit colouration (Saure, 1990) with the largest differences in the lower, inner part of the canopy (Fig. 5), which is crucial since colouration is usually poor due to insufficient availability of light (Blanke, 2015). The better red colouration (30°hue) (Fig. 5) was due to improvements on the shaded side of the fruit as a result of increased diffuse light through the reflective ground cover (Fig. 2), with negligible changes on the sun-exposed side. Our results have shown that even a well colouring mutant like 'Braeburn Mariri Red' is susceptible to fruit colour improvement under favourable temperature differences in the last ripening period in 2016 with night temperatures beneath 10 °C and day temperatures over 25 °C. Since a single night is sufficient to initiate the transcription of the *MYB10* gene and induce anthocyanin synthesis, several nights in the September of the year 2016 achieved a high stimulation of anthocyanin synthesis (Ban et al., 2007; Lin-Wang et al., 2011; Bassett and Glenn, 2014).

5. Conclusions

Improvements of fruit colouration (anthocyanin synthesis) on the shaded side of fruit and fruit in the inner lower part of the tree canopy were the result of light interception. Light reflection was improved by 1.5 to 3.9 times in autumn at 50 °N by the use of the reflective ground cover Lumilys® compared with the grassed alleyway in the orchard. This was in a situation, where the light loss caused by hailnets ranged from 0% to 5% on a sunny day and 2% to 10% on an overcast day.

The coloured visualization from orange (intense light), yellow (medium) to green (low light intensity) as a result of the individual PAR measurements inside the canopy showed how the reflective mulch influences the light penetration into the different parts of the tree canopy, especially in the lower, inner part of the canopy, explaining the improved fruit colouration (anthocyanin synthesis).

Author statement

Authors confirm no conflict of interest and no soft, external funding for the project

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jplph.2018.12.008.

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